Supplemental Information S1. Overview of Current and Prospective Anti-Biofilm Strategies					
Types	**Biofilm component**	**Biofilm phase**	**State of Development[&]**	**Pros**	**Cons**
Agents					
Antibiotics[1,2]	Microbial cell	All stages	Clinical	*Well understood. *Novel combinations promising. *Many can be combined with local delivery.	*Resistance. *Cytotoxicity. *Not necessarily effective against dormant populations. *Some have limited penetration into biofilm EPS.
Antimicrobial peptides[3]	Microbial cell	All stages	Pre-clinical	*Small molecules easily engineered for optimization. *Membrane physical disruption reduces probability of resistance. *Broad-spectrum *Species-specific targeting possible.	*Charge may limit transport through biofilm EPS. *Potential proteolytic degradation. *pH may affect activity *Delivery to infected site
Antimicrobial oligonucleotides[4,5]	Microbial cell	Early/Mature biofilm	In vivo	*Small molecules easily engineered for optimization.	*Charge may limit transport through biofilm EPS. *Potential binding with eDNA. *Delivery to infected site *Potential degradation by nucleases
Nanoparticles (inorganic, organic, hybrid)[6-8]	Microbial cell, EPS	All stages	In vivo, pre-clinical, clinical†	*Readily functionalized. *Intrinsic bioactivity combined with drug-delivery capacity. *Small size allows transport into the EPS. *Triggered (pH, O_2) mechanism possible for	*Charge may limit penetration into the biofilm EPS. *Properties affected by biological fluids. *Delivery to the infected site. *Cytotoxicity.

				on demand treatment.	
Other antimicrobials/oxidizers/ antiseptics[1]	Microbial cell	All stages	Clinical	*Physical mode of action not requiring cellular activity. *Broad-spectrum.	*Lack of targeting specificity. * Restricted transport into biofilm EPS. *Cytotoxicity. *Reactive species neutralized by EPS.
Persisters/dormant cells targeting [9]	Microbial cell	Early/Mature biofilm	In vivo	*Specifically targeted to non-growing populations.	*Resistance (not well understood). *Delivery to infected site and transport into the biofilm.
Antibody/Vaccines[1]	Microbial cell, EPS	Initial attachment, early biofilm	In vivo	*Targeted to specific pathogens.	*Restricted transport into biofilm EPS. *Strain replacement. *Disruption of commensal populations.
Adhesin inhibitors/binding[10]	EPS	Initial attachment	Pre-clinical	*Prevention preferable to treatment.	*Symptomatic infections have established biofilms. *Interaction with host components. *Delivery to at risk or infected site.
Bacteriophages[7]	Microbial cell	Early/Mature biofilm	In vivo	*Highly specific and small size to enter biofilm EPS.	*Strain replacement. *Delivery to infected site and transport into the biofilm.
Detergent/Surfactant irrigants[1,11]	Microbial cell, EPS	All stages	Clinical	*Disruption not dependent of killing cells.	*Not all biofilm removed. *Release of pathogens may result in recolonization and

				*Active on dormant cells. *Readily combined for multimodal therapeutics.	acute infection.
Dispersal Inducers[12,13]	Microbial cell	Mature biofilm	In vitro, In vivo, pre-clinical, clinical	*Manipulating natural processes might be less likely to develop resistance.	*Release of pathogens may result in recolonization and acute infection. *Only portions of the biofilms are released. *Cytotoxicity. *Delivery to infected site and transport into the biofilm.
Degradative Enzymes[14,15]	EPS	Early/Mature biofilm	Clinical, pre-clinical	*Disruption not dependent on killing cells. *Weaken biofilm physical structure; facilitate mechanical removal/mass transport. *Disrupt pathogenic microenvironment. *Cell activity not required. *Readily combined with irrigants and shear.	*Not all biofilm removed, possibly due to complex EPS chemistry and physical structure. *Release of pathogens may result in recolonization and acute infection. *Delivery to infected site *No, or limited, antimicrobial activity. *Cytotoxicity
EPS synthesis inhibitors[1]	EPS	Initial attachment, early biofilm	In vivo, In vitro	*Prevention of early biofilm formation and EPS protection. *Readily combined with antimicrobials	*Most infections have established biofilms by the time they are symptomatic. *EPS chemistry and structure highly complex. *Delivery to at risk or infected site.

Natural products[16]	Microbial cell, EPS	All stages	In vivo, clinical	*Selected for broad-range of bioactivity (from enzyme inhibitors to antimicrobials). *Chemical diversity with drug-like properties *Multi-mode of action	*Resistance. *Complex chemistry and isolation procedures. *Chemical composition variability. *Target identification *Cytotoxicity.
Photodynamic substances[17]	Microbial cell	Early/Mature biofilm	In vivo	*Controlled bioactivation options. *On demand activity.	*Light source access required *Delivery of materials to infected site and transport into biofilm. *EPS may protect cells deeper down.
Metabolic interference[12]	Microbial cell	Early/Mature biofilm	In vivo, In vitro	*Community manipulation against pathogens. *Disrupt pathogenic environment (pH). *Manipulating metabolism less likely to develop resistance. *Can trigger disassembly	*Requires specific metabolizing microbes. *Substrate delivery to and transport into biofilms. *Potential substrate utilization by host. *Release of pathogens may result in recolonization and acute infection.
QS inhibitors[18]	Microbial cell	All stages	Pre-clinical, In vivo	*Manipulating natural pathways less likely to develop resistance. *Biofilm inhibition and biofilm dispersal	*Dependent on growth cycle and nutrient source. *Signals can be washed away or sequestered in the EPS matrix of established biofilm *Complexity of signaling network.

Probiotics[19]	Microbial cell	Initial attachment, early biofilm	In vitro, Pre-clinical (in oral), clinical†	*Community manipulation against pathogens *Concept proven in gut and vaginal biofilms.	* Establishment of probiotic species in oral (and other established) microbiota challenging *Long-term effects unknown
Physical/Electric					
Electric currents/fields[20,21]	Microbial cell, EPS	Early/Mature biofilm	Clinical, pre-clinical	*Projected through induction or connected wires. *On demand antimicrobial generation. *Also promote wound healing.	*Electrochemistry of body fluids not well understood. *Heating of tissue. *Delivery of fields and currents to deep tissue. *Cytotoxicity.
Transducer/pressure waves[22]	Microbial cell, EPS	Early/Mature biofilm	In vivo, pre-clinical	*Readily projected through skin and soft tissue. *Local delivery. *Physical action reduces probability of resistance.	*Limited targeting. *Influence of pressure waves on viscoelastic biofilms not well understood. *Local delivery (i.e. shockwave) limited to small and accessible areas. *Heating cytotoxic effects.
Interfacial tension (microbubbles/droplets)[23]	Microbial cell, EPS	Early/Mature biofilm	Pre-clinical	*Physical action reduces probability of resistance. *Readily combined with irrigants and shear.	*Accessibility. *Biofilm viscoelasticity can resist removal. *Residual cells may remain.
Shear stress[22]	Microbial cell, EPS	Early/Mature biofilm	Clinical	*Physical action reduces probability of resistance. *Readily combined with antimicrobials or nanoparticles.	*Accessibility. *Biofilm viscoelasticity can resist removal. *Possible spread of biofilm if not used in combination with antimicrobial agents.
Non-thermal (cold) plasma[24]	Microbial cell	Early/Mature biofilm	In vivo	*Antimicrobials generated locally. *High level of	*Accessibility of plasma. *Biofilm EPS may protect cells deeper down.

				oxidation/reactive species renders resistance unlikely.	*Response to plasma is species-dependent. *Highly localized.
Photothermal activation[24]	Microbial cell	Early/Mature biofilm	In vitro	*Antimicrobial activity can be controlled locally. *Can be readily combined with surface modifications.	*Delivery to infected site and transport into biofilm. *Accessibility of light. *Biofilm EPS may protect cells.
Delivery Systems					
Bone cements [25]	Microbial cell	Initial attachment, mature biofilm	Clinical	*High concentrations of antibiotics maintained at site of local infection for extended periods. *Prophylactic use.	*Antimicrobial cytotoxicity. *Development of resistance.
Rinsing fluid/Irrigators [26,27]	Microbial cell, EPS	Mature biofilm	Clinical	*Can be readily combined with antimicrobial agents.	*Accessibility. *Biofilm viscoelasticity can resist removal
Surfaces [28,29]	Microbial cell	Initial attachment	Clinical	*Prevention more effective than treatment. *Access not required after implantation. *Can be targeted to those surfaces prone of biofilm infection.	*Bacteria have non-specific attachment mechanisms. *Surfaces masked by dead biofilm or host components. *Stability of surface coatings. *Finite antimicrobial reservoir/long-term effects
Nanocarriers (nanoparticles/liposomes) [6]	Microbial cell, EPS	Early/Mature biofilm	In vivo, pre-clinical, clinical	*Readily functionalized. *Small size allows transport into the EPS *Carry/release different drug combinations *Triggered (pH, O_2) mechanism possible for on demand drug-release.	*Charge may limit penetration into the biofilm EPS. *Delivery to the infected site *Properties affected by biological fluids. *Cytotoxicity *Prolonged retention needed for optimal drug release

[&]Specifically against biofilms

†used clinically to treat other conditions

ð Clinical - already a licensed product available to patients;
Pre-clinical - currently in human trials
In vivo - currently in animal model
In vitro - encompassing basic (polystyrene plate) to advanced biofilm research (i.e. co-culture, explant tissue, patient samples)

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